INVITRO AND *INVIVO* EVALUATION OF ANTICANCER ACTIVITY OF DIFFERENT EXTRACTS OF *IPOMOEA BATAUS* STEM

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY,
CHENNAI– 600 032

In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
BRANCH –VI - PHARMACOLOGY

Submitted by
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MAY - 2017

CERTIFICATE

This is to certify that the M.Pharm Dissertation entitled **"Invitro And *Invivo* Evaluation Of Different Extract Of *ipomoea batatus* stem"**being submitted to The TamilNadu Dr. M.G.R Medical University, Chennai was carried out by **Ms. ANOOPA WILSON** to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfillment for the degree of **MASTER OF PHARMACY IN PHARMACOLOGY** is a bonafied work carried out by candidate under my guidance and supervision in the Department of Pharmacology , Karpagam college of Pharmacy Coimbatore – 32.

I have fully satisfied with her performance and work. I have forwarded this dissertation work for evaluation.

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I have fully satisfied with her performance and work. I have forwarded this dissertation work for evaluation.

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DECLARATION

I hereby declare that this dissertation **"*Invitro* And *Invivo* Evaluation Of Different Extracts of *Ipomoea Batatus* stem"** submitted by the candidate, in partial fulfillment of requirements for the degree of **MASTER OF PHARMACY IN PHARMACOLOGY** to The Tamil Nadu Dr.M.G.R Medical University, Chennai is the result of my original and independent research work carried out under the guidance of **Prof .G.NAGARAJA PERUMAL., M.Pharm.,(Ph.D)** Professor & Head Department of Pharmacology ,Karpagam College of Pharmacy, Coimbatore -32,& Co Guide **Dr Hashim K.M.,UWIN LIFE SCIENCE,** during the acdemic year 2016-2017.

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EVALUATION CERTIFICATE

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Examination centre :

Date :

InternalExaminer Convenor of Examination

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DEDICATED TO MY BELOVED PARENTS, TEACHERS AND ALMIGHTY

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ANOOPA WILSON

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CONTENTS

CHAPTER I

1.INTRODUCTION

1.1 CANCER

Cancer is a diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.[1]&[2] Not all tumors are cancerous; benign tumors do not spread to other parts of the body.[2] Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss and a change in bowel movements. While these symptoms may indicate cancer, they may have other causes.[3] Over 100 cancers affect humans.[2]

Many cancers can be prevented by not smoking, maintaining a healthy weight, not drinking too much alcohol, eating plenty of vegetables, fruits and whole grains, vaccination against certain infectious diseases, not eating too much processed and red meat, and avoiding too much sunlight exposure.[9]&[10] Early detection through screening is useful for cervical and colorectal cancer.[11] The benefits of screening in breast cancer are controversial.[11]&[12] Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy.[1]&[13] Pain and symptom management are an important part of care. Palliative care is particularly important in people with advanced disease.[1] The chance of survival depends on the type of cancer and extent of disease at the start of treatment.[6] In children under 15 at diagnosis the five-year survival rate in the developed world is on average 80%.[14] For cancer in the United States the average five-year survival rate is 66%.[15]

Worldwide approximately 18% of cancer deaths are related to infectious diseases. This proportion ranges from a high of 25% in Africa to less than 10% in the developed world.[5] Viruses are the

usual infectious agents that cause cancer but cancer bacteria and parasites may also play a role.

In 2012 about 14.1 million new cases of cancer occurred globally (not including skin cancer other than melanoma).[6] It caused about 8.2 million deaths or 14.6% of human deaths.[6]&[16] The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer and stomach cancer. In females, the most common types are breast cancer, colorectal cancer, lung cancer and cervical cancer.[6] If skin cancer other than melanoma were included in total new cancers each year it would account for around 40% of cases.[17]&[18] In children, acute lymphoblastic leukaemia and brain tumors are most common except in Africa where non-Hodgkin lymphoma occurs more often.[14] In 2012, about 165,000 children under 15 years of age were diagnosed with cancer. The risk of cancer increases significantly with age and many cancers occur more commonly in developed countries.[6] Rates are increasing as more people live to an old age and as lifestyle changes occur in the developing world.[19] The financial costs of cancer were estimated at $1.16 trillion US dollars per year as of 2010.[20]

Cancers are a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body.[1]&[2] They form a subset of neoplasms. A neoplasm or tumor is a group of cells that have undergone unregulated growth and will often form a mass or lump, but may be distributed diffusely.[21][22]

All tumor cells show the six hallmarks of cancer. These characteristics are required to produce a malignant tumor. They include:[23]

- Cell growth and division absent the proper signals
- Continuous growth and division even given contrary signals
- Avoidance of programmed cell death
- Limitless number of cell divisions

- Promoting blood vessel construction
- Invasion of tissue and formation of metastases[24]

The progression from normal cells to cells that can form a detectable mass to outright cancer involves multiple steps known as malignant progression.[24]&[25]

When cancer begins, it produces no symptoms. Signs and symptoms appear as the mass grows or ulcerates. The findings that result depend on the cancer's type and location. Few symptoms are specific. Many frequently occur in individuals who have other conditions. Cancer is a "great imitator". Thus, it is common for people diagnosed with cancer to have been treated for other diseases, which were hypothesized to be causing their symptoms.[26]

People may become anxious or depressed post-diagnosis. The risk of suicide in people with cancer is approximately double.[27]

Local symptoms may occur due to the mass of the tumor or its ulceration. For example, mass effects from lung cancer can block the bronchus resultingin cough or pneumonia; esophagealcancer can cause narrowing of the esophagus, making it difficult or painful to swallow; and colorectal cancer may lead to narrowing or blockages in the bowel, affecting bowel habits. Masses in breasts or testicles may produce observable lumps. Ulceration can cause bleeding that, if it occurs in the lung, will lead to coughing up blood, in the bowels to anemia or rectal bleeding, in the bladder to blood in the urine and in the uterus to vaginal bleeding. Although localized pain may occur in advanced cancer, the initial swelling is usually painless. Some cancers can cause a buildup of fluid within the chest or abdomen.[26]

1.1.2Systemic symptoms

General symptoms occur due to effects that are not related to direct or metastatic spread. These may include: unintentional

weight loss, fever.[28] Hodgkin disease,leukemia and cancers of the liver or kidney can cause a persistent fever.[26]

Some cancers may cause specific groups of systemic symptoms, termed paraneoplastic phenomena.[26]

1.1.2.3 Mechanism Of Cancer Cell Formation And Spreading

Cancer can spread from its original site by local spread, lymphatic spread to regional lymph nodes or by hematogenous spread via the blood to distant sites, known as metastasis. When cancer spreads by a hematogenous route, it usually spreads all over the body. However, cancer 'seeds' grow in certain selected site only ('soil') as hypothesized in the soil and seed hypothesis of cancer metastasis. The symptoms of metastatic cancers depend on the tumor location and can include enlarged lymph nodes (which can be felt or sometimes seen under the skin and are typically hard), enlarged liver or enlarged spleen, which can be felt in the abdomen, pain or fracture of affected bones and neurological symptoms.[26]

1.2 Causes

The majority of cancers, some 90–95% of cases, are due to environmental factors. The remaining 5–10% are due to inherited genetics. Environmental, as used by cancer researchers, means any cause that is not inherited genetically, such as lifestyle, economic and behavioral factors and not merely pollution.[29] Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation, stress.

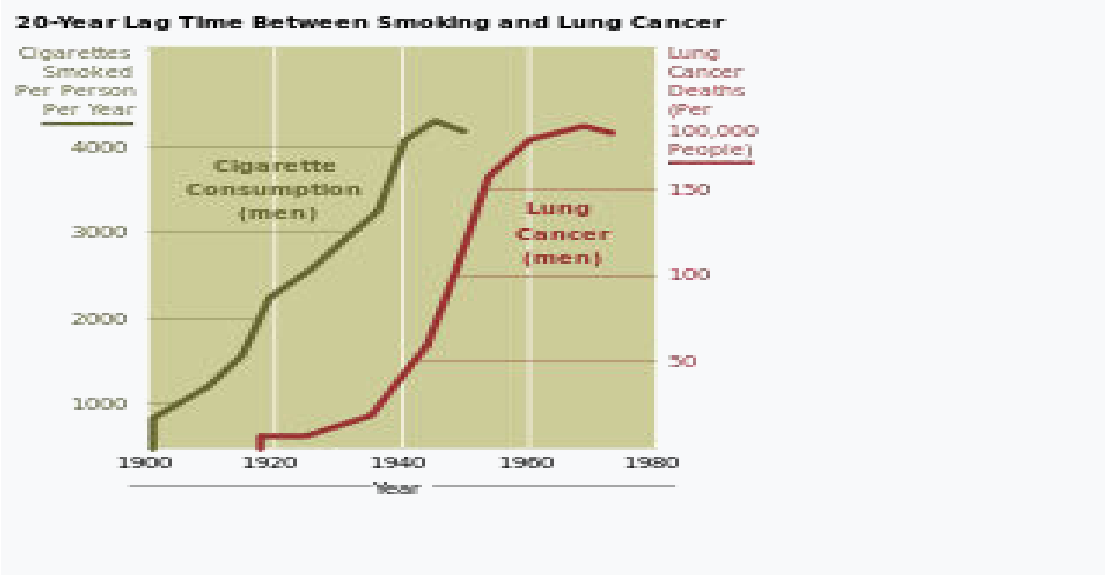
Tobacco use is the cause of about 22% of cancer deaths.[1] Another 10% is due to obesity, poor diet, lack of physical activity and drinking alcohol.[1]&[4] Other factors include certain infections,

exposure to ionizing radiation and environmental pollutants.[5] It is due to infections such as hepatitis B, hepatitis C and human papillomavirus (HPV).[1] These factors act, at least partly, by changing the genes of a cell. Typically many genetic changes are required before cancer develops.[6] Approximately 5–10% of cancers are due to inherited genetic defects from a person's parents.[7] Cancer can be detected by certain signs and symptoms or screening tests.[1] It is then typically further investigated by medical imaging and confirmed by biopsy.[8]

It is not generally possible to prove what caused a particular cancer because the various causes do not have specific fingerprints. For example, if a person who uses tobacco heavily develops lung cancer, then it was probably caused by the tobacco use, but since everyone has a small chance of developing lung cancer as a result of air pollution or radiation, the cancer may have developed for one of those reasons. Excepting the rare transmissions that occur with pregnancies and occasional organ donors, cancer is generally not a transmissible disease.[30]

1.21 Chemicals

Figure No.1: Lag time between Smoking and Lung Cancer



The incidence of lung cancer is highly correlated with smoking.

Exposure to particular substances have been linked to specific types of cancer. These substances are called carcinogens.

Tobacco smoke, for example, causes 90% of lung cancer.[31] It also causes cancer in the larynx, head, neck, stomach, bladder, kidney, esophagus and pancreas.[32] Tobacco smoke contains over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons.[33]

Tobacco is responsible about one in five cancer deaths worldwide [33] and about one in three in the developed world.[34].Lung cancer death rates in US have mirrored smoking patterns, with increases in smoking followed by dramatic increases in lung cancer death rates and, more recently, decreases in smoking rates since the 1950s followed by decreases in lung cancer death rates in men since 1990.[35]&[36]

In Western Europe, 10% of cancers in males and 3% of cancers in females are attributed to alcohol exposure, especially liver and digestive tract cancers.[37] Cancer from work-related substance exposures may cause between 2 and 20% of cases, [38] causing at least 200,000 deaths. Cancers such as lung cancer and mesothelioma can come from inhaling tobacco smoke or asbestos fibers, or leukemia from exposure to benzene.[39]

1.2.2 Life style Changes

Diet, physical inactivity and obesity are related to up to 30–35% of cancer deaths.[5][40] In the United States excess body weight is associated with the development of many types of cancer and is a factor in 14–20% of cancer deaths.[40] A UK study including data on over 5 million people showed higher body mass index to be related to at least 10 types of cancer and responsible for around 12,000

cases each year in that country.[41] Physical inactivity is believed to contribute to cancer risk, not only through its effect on body weight and also through negative effects on the immune system and endocrine system. More than half of the effect from diet is due to overnutrition (eating too much), rather than from eating too few vegetables or other healthful foods.

Some specific foods are linked to specific cancers. A high-salt diet is linked to gastric cancer.[42] Aflatoxin B1, a frequent food contaminant, causes liver cancer. Betel nut chewing can cause oral cancer.[42] National differences in dietary practices may partly explain differences in cancer incidence. For example, gastric cancer is more common in Japan due to its high-salt diet while colon cancer is more common in the United States. Immigrant cancer profiles develop mirror that of their new country, often within one generation.[44]

1.2.3. Infection

Oncoviruses (viruses that can cause cancer) include human papillomavirus (cervical cancer), Epstein–Barr virus (B-cell lymphoproliferativedisease and nasopharyngealcarcinoma), Kaposi's sarcoma herpesvirus (Kaposi's sarcoma and primary effusion lymphomas), hepatitis B and hepatitis C viruses (hepatocellular carcinoma) and human T-cell leukemia virus-1 (T-cell leukemias).

1.2.4. Radiation

Up to 10% of invasive cancers are related to radiation exposure, including both ionizing radiation and non-ionizing ultraviolet radiation.[5] Additionally, the majority of non-invasive cancers are non-melanoma skin cancers caused by non-ionizing ultraviolet radiation, mostly from sunlight. Sources of ionizing radiation include medical imaging and radon gas.

Ionizing radiation is not a particularly strong mutagen.[48] Residential exposure to radon gas, for example, has similar cancer risks as passive smoking. Radiation is a more potent source of cancer when combined with other cancer-causing agents, such as radon plus tobacco smoke. Radiation can cause cancer in most parts of the body, in all animals and at any age..

Medical use of ionizing radiation have small growing source of radiation-induced cancers. Ionizing radiation may be used to treat other cancers, but this may, in some cases, induce a second form of cancer.[48] It is also used in some kinds of medical imaging.[49]

Prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies. Clear evidence establishes ultraviolet radiation, especially the non-ionizing medium wave UVB, as the cause of most non-melanoma skin cancers, which are the most common forms of cancer in the world.[50]

Non-ionizing radio frequency radiation from mobile phones, electric power transmission and other similar sources have been described as a possible carcinogen by the World Health Organization's International Agency for Research on Cancer.[51] However, studies have not found a consistent link between mobile phone radiation and cancer risk.[52]

Some substances cause cancer primarily through their chemical rather than physical, effects. A prominent example of this is prolonged exposure to asbestos, naturally occurring mineral fibers that are a major cause of mesothelioma (cancer of the serous membrane) usually the serous membrane surrounding the lungs. Other substances in this category, including both naturally occurring and synthetic asbestos-like fibers, such as wollastonite, attapulgite, glass wool and rock wool, are believed metallic cobalt and nickel and crystallinesilica (quartz, cristobalite

and tridymite). Usually, physical carcinogens must get inside the body (such as through inhalation) and require years of exposure to produce cancer.[55] Children and adolescents are twice as likely to develop radiation-induced leukemia as adults; radiation exposure before birth has ten times the effect

Physical trauma resulting in cancer is relatively rare. Claims that breaking bones resulted in bone cancer, for example, have not been proven. Similarly, physical trauma is not accepted as a cause for cervical cancer, breast cancer or brain cancer. One accepted source is frequent, long-term application of hot objects to the body. It is possible that repeated burns on the same part of the body, such as those produced by kanger and kairo heaters (charcoal hand warmers), may produce skin cancer, especially if carcinogenic chemicals are also present. Frequent consumption of scalding hot tea may produce esophageal cancer.[56] Generally, it is believed that cancer arises, or a pre-existing cancer is encouraged, during the process of healing, rather than directly by the trauma. However, repeated injuries to the same tissues might promote excessive cell proliferation, which could then increase the odds of a cancerous mutation.[56]

Chronic inflammation has been hypothesized to directly cause mutation.[56][57] Inflammation can contribute to proliferation, survival, angiogenesis and migration of cancer cells by influencing the tumor microenvironment.[58]&[59] Oncogenes build up an inflammatory pro-tumorigenic microenvironment.[60]

1.2.5 Hormones

Some hormones play a role in the development of cancer by promoting cell proliferation.[61] Insulin-like growth factors and their binding proteins play a key role in cancer cell proliferation, differentiation, apoptosis, suggesting possible involvement in carcinogenesis.[62]

Hormones are important agents in sex-related cancers, such as cancer of the breast, endometrium, prostate, ovary, testis and also of thyroid cancer and bone cancer. For example, the daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. These higher hormone levels may explain their higher risk of breast cancer, even in the absence of a breast-cancer gene. Similarly, men of African ancestry have significantly higher levels of testosterone than men of European ancestry and have a correspondingly higher level of prostate cancer. Men of Asian ancestry, with the lowest levels of testosterone-activating androstanediolglucuronide, have the lowest levels of prostate cancer.[61]

Other factors are relevant: obese people have higher levels of some hormones associated with cancer and a higher rate of those cancers. Women who take hormone replacement therapy have higher risk of developing cancers associated with those hormones. On the other hand, people who exercise far more than average have lower levels of these hormones and lower risk of cancer. Osteosarcoma may be promoted by growth hormones. Some treatments and prevention approaches leverage this cause by artificially reducing hormone levels and thus discouraging hormone-sensitive cancers.[61] Cancer is fundamentally disease of tissue growth regulation. In order for a normal cell to transform into a cancer cell, the genes that regulate cell growth and differentiation must be altered.[65]

The affected genes are divided into two broad categories. Oncogenes are genes that promote cell growth and reproduction. Tumor suppressor genes are genes that inhibit cell division and survival. Malignant transformation can occur through the formation of novel oncogenes, the inappropriate over-expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in multiple

genes are required to transform a normal cell into a cancer cell.[66]

Genetic changes can occur by different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic DNA.

Large-scale mutations involve the deletion or gain of a portion of a chromosome. Genomic amplification occurs when a cell gains copies (often 20 or more) of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material. Translocation occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location. A well-known example of this is the Philadelphia chromosome, or translocation of chromosomes 9 and 22, which occurs in chronic myelogenous leukemia and results in production of the BCR-abl fusion protein, an oncogenic tyrosine kinase.

Small-scale mutations include point mutations, deletions, and insertions, which are in the promoter region of a gene and affect its expression, or may occur in the gene's coding sequence and alter the function or stability of its protein product. Disruption of a single gene may also result from integration of genomic material from a DNA virus or retrovirus, leading to the expression of viral oncogenes in the affected cell and its descendants.

Replication of the data contained within the DNA of living cells will probabilistically result in some errors (mutations). Complex error correction and prevention is built into the process and safeguards the cell against cancer. If a significant error occurs, the damaged cell can self-destruct through programmed cell death, termed apoptosis. If the error control processes fail, then the mutations will survive and be passed along to daughter cells.

Some environments make errors more likely to arise and propagate. Such environments can include the presence of

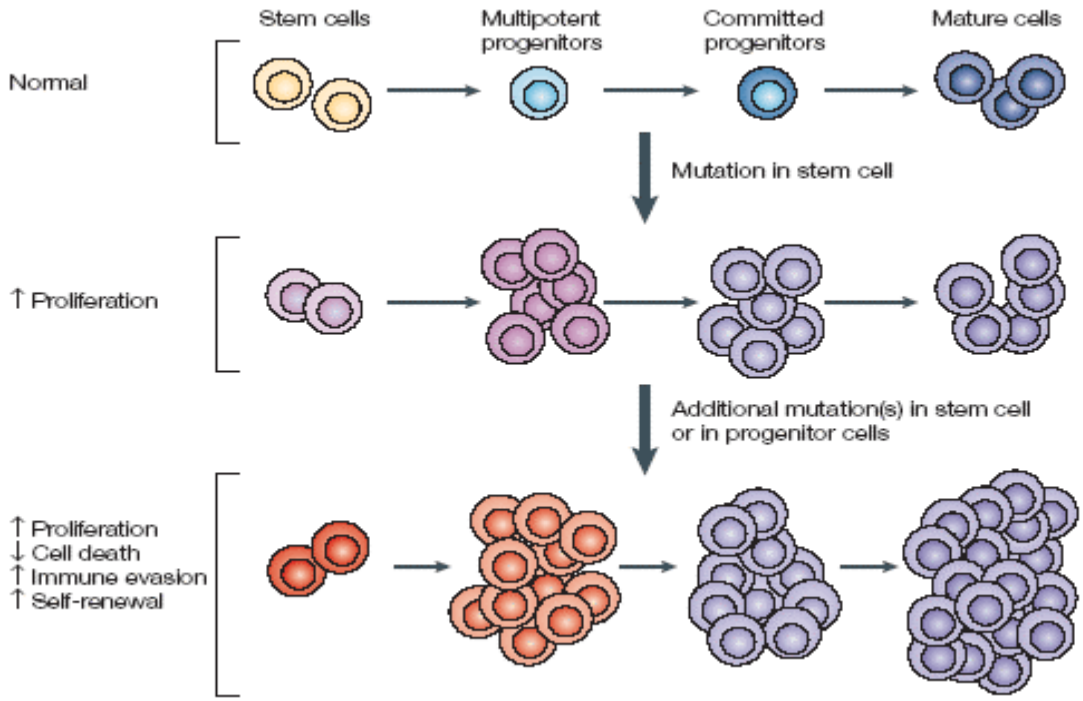
disruptive substances called carcinogens, repeated physical injury, heat, ionising radiation or hypoxia.[67]

The errors that cause cancer are self-amplifying and compounding, for example:

- A mutation in the error-correcting machinery of a cell might cause that cell and its children to accumulate errors more rapidly.
- A further mutation in an oncogene might cause the cell to reproduce more rapidly and more frequently than its normal counterparts.
- A further mutation may cause loss of a tumor suppressor gene, disrupting the apoptosis signaling pathway and immortalizing the cell.
- A further mutation in the signaling machinery of the cell might send error-causing signals to nearby cells.

The transformation of a normal cell into cancer is akin to a chain reaction caused by initial errors, which compound into more severe errors, each progressively allowing the cell to escape more controls that limit normal tissue growth. This rebellion-like scenario is an undesirable survival of the fittest, where the driving forces of evolution work against the body's design and enforcement of order. Once cancer has begun to develop, this ongoing process, termed clonal evolution, drives progression towards more invasive stages.[68] Clonal evolution leads to intra-tumour heterogeneity (cancer cells with heterogeneous mutations) that complicates designing effective treatment strategies

Figure No:2 Cell Proliferation



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Characteristic abilities developed by cancers are divided into categories, specifically evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, sustained angiogenesis, limitless replicative potential, metastasis, reprogramming of energy metabolism and evasion of immune destruction.[24]&[25]

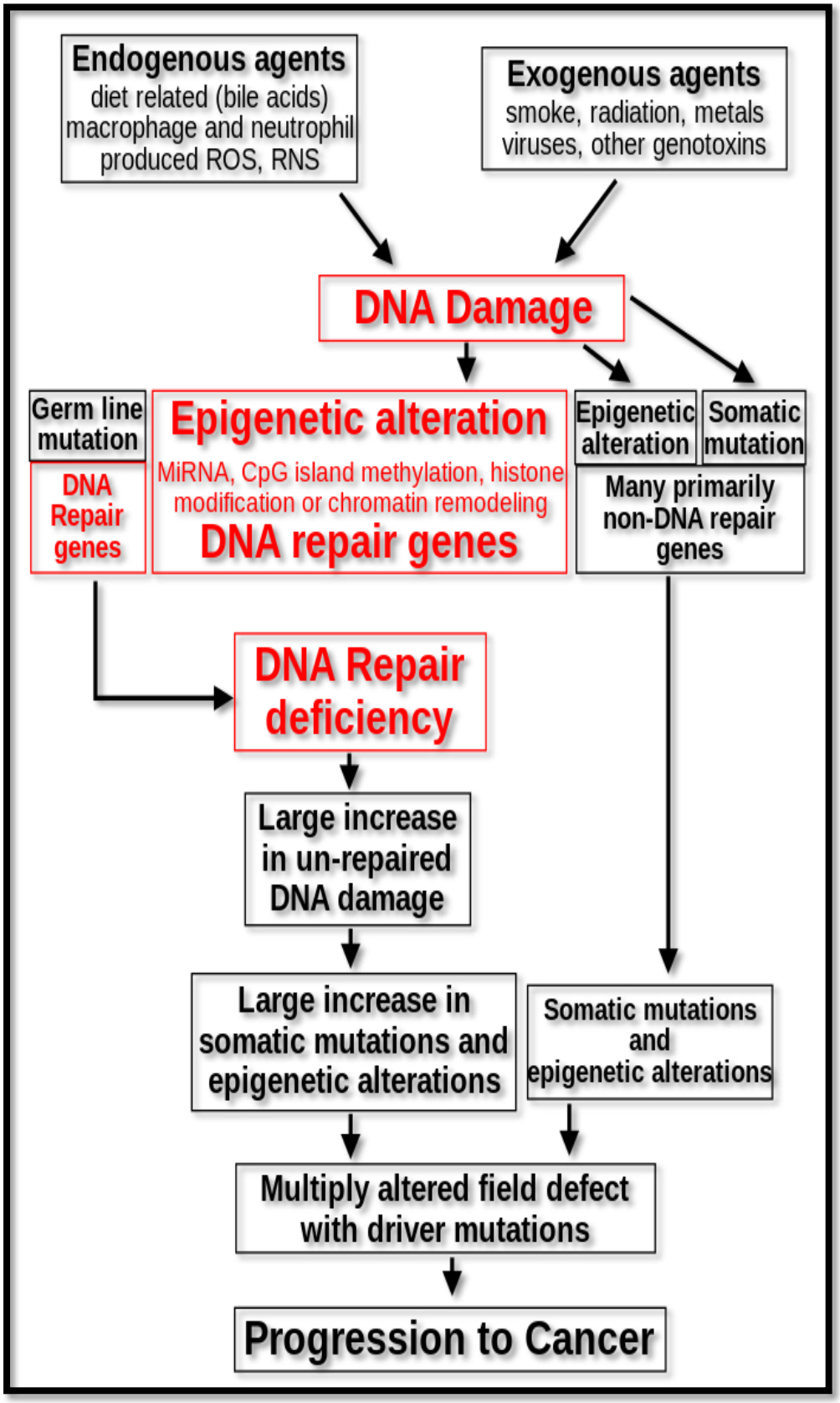
The classical view of cancer is a set of diseases that are driven by progressive genetic abnormalities that include mutations in tumor-suppressor genes and oncogenes and chromosomal abnormalities. Later epigenetic alterations' role was identified.[69]

Epigenetic alterations refer to functionally relevant modifications to the genome that do not change the nucleotide sequence. Examples of such modifications are changes in DNA methylation (hypermethylation and hypomethylation), histone

modification[70] and changes in chromosomal architecture (caused by inappropriate expression of proteins such as HMGA2 or HMGA1).[71] Each of these alterations regulates gene expression without altering the underlying DNA sequence. These changes may remain through cell divisions, last for multiple generations and can be considered to be epimutations (equivalent to mutations).

Epigenetic alterations occur frequently in cancers. As an example, one study listed protein coding genes that were frequently altered in their methylation in association with colon cancer. These included 147 hypermethylated and 27 hypomethylated genes. Of the hypermethylated genes, 10 were hypermethylated in 100% of colon cancers and many others were hypermethylated in more than 50% of colon cancers.[72]

Figure No:3


Endogenous agents
diet related (bile acids)
macrophage and neutrophil
produced ROS, RNS
Exogenous agents
smoke, radiation, metals
viruses, other genotoxins
DNA Damage
Germ line mutation
DNA Repair genes
Epigenetic alteration
MiRNA, CpG island methylation, histone modification or chromatin remodeling
DNA repair genes
Epigenetic alteration
Somatic mutation
Many primarily non-DNA repair genes
DNA Repair deficiency
Large increase in un-repaired DNA damage
Large increase in somatic mutations and epigenetic alterations
Somatic mutations and epigenetic alterations
Multiply altered field defect with driver mutations
Progression to Cancer

While epigenetic alterations are found in cancers, the epigenetic alterations in DNA repair genes, causing reduced expression of DNA repair proteins, may be of particular importance. Such alterations are thought to occur early in progression to cancer and to be a likely cause of the genetic instability characteristic of cancers.[73],[74],[75]&[76]

Reduced expression of DNA repair genes disrupts DNA repair. This is shown in the figure at the 4th level from the top. (In the figure, red wording indicates the central role of DNA damage and defects in DNA repair in progression to cancer.) When DNA repair is deficient DNA damage remains in cells at a higher than usual level (5th level) and cause increased frequencies of mutation and/or epimutation (6th level). Mutation rates increase substantially in cells defective in DNA mismatch repair[77]&[78] or in homologous recombinational repair (HRR).[79] Chromosomal rearrangements and aneuploidy also increase in HRR defective cells.[80]

Higher levels of DNA damage cause increased mutation (right side of figure) and increased epimutation. During repair of DNA double strand breaks, or repair of other DNA damage, incompletely cleared repair sites can cause epigenetic gene silencing.[81][82]

Deficient expression of DNA repair proteins due to an inherited mutation can increase cancer risks. Individuals with an inherited impairment in any of 34 DNA repair genes (see article DNA repair-deficiency disorder) have increased cancer risk, with some defects ensuring a 100% lifetime chance of cancer (e.g. p53 mutations).[83] Germ line DNA repair mutations are noted on the figure's left side. However, such germline mutations (which cause highly penetrant cancer syndromes) are the cause of only about 1 percent of cancers.[84]

In sporadic cancers, deficiencies in DNA repair are occasionally caused by a mutation in a DNA repair gene but are much more frequently caused by epigenetic alterations that reduce or silence

expression of DNA repair genes. This is indicated in the figure at the 3rd level. Many studies of heavy metal-induced carcinogenesis show that such heavy metals cause a reduction in expression of DNA repair enzymes, some through epigenetic mechanisms. DNA repair inhibition is proposed to be a predominant mechanism in heavy metal-induced carcinogenicity. In addition, frequent epigenetic alterations of the DNA sequences code for small RNAs called microRNAs (or miRNAs). miRNAs do not code for proteins, but can "target" protein-coding genes and reduce their expression.

Cancers usually arise from an assemblage of mutations and epimutations that confer a selective advantage leading to clonal expansion.[85]

Cancer is becoming a high profile disease in developed and developing worlds. In 2007 the WHO published that in 2005, 7.6 million people died from cancer related diseases with the majority of these people living in low-income countries . In the United States cancer is the cause of 1 in 4 deaths and in 2010 it was estimated there were over 1.5 million new cases of cancer . Cancer Research UK said in 2012 14.1 million adults were diagnosed with cancer and 8.2 million people were killed by cancer globally . Therefore, the demand for a cure and the prevention of cancer is extremely high.

1.2.6. Dietary

While many dietary recommendations have been proposed to reduce cancer risks, the evidence to support them is not definitive.[9]&[93] The primary dietary factors that increase risk are obesity and alcohol consumption. Diets low in fruits and vegetables and high in red meat have been implicated but reviews and meta-analyses do not come to a consistent conclusion. [94]&[95] A 2014 meta-analysis find no relationship between fruits and vegetables and cancer.[96] Coffee is associated with a reduced risk

of liver cancer.[97] Studies have linked excess consumption of red or processed meat to an increased risk of breast cancer, colon cancer and pancreatic cancer, a phenomenon that could be due to the presence of carcinogens in meats cooked at high temperatures. [98]&[99] In 2015 the IARC reported that eating processed meat (e.g., bacon, ham, hot dogs, sausages) and, to a lesser degree, red meat was linked to some cancers.[100]&[101] Dietary recommendations for cancer prevention typically include an emphasis on vegetables, fruit, whole grains and fish and an avoidance of processed and red meat (beef, pork, lamb), animal fats and refined carbohydrates. [9]&[93]

1.2.7. Medication

Medications can be used to prevent cancer in a few circumstances.[102] In the general population, NSAIDs reduce the risk of colorectal cancer; however, due to cardiovascular and gastrointestinal side effects, they cause overall harm when used for prevention.[103] Aspirin has been found to reduce the risk of death from cancer by about 7%. [104] COX-2 inhibitors may decrease the rate of polyp formation in people with familial adenomatous polyposis; however, it is associated with the same adverse effects as NSAIDs.[105] Daily use of tamoxifen or raloxifene reduce the risk of breast cancer in high-risk women.[106] The benefit versus harm for 5-alpha-reductase inhibitor such as finasteride is not clear.[107]

Vitamins are not effective at preventing cancer,[108] although low blood levels of vitamin D are correlated with increased cancer risk.[109]&[110] People who have cancer are also at a high risk of developing vitamin D deficiency.[111] Whether this relationship is causal and vitamin D supplementation is protective is not determined.[112] Beta-carotene supplementation increases lung cancer rates in those who are high risk.[113] Folic acid supplementation is not effective in preventing colon cancer

and may increase colon polyps.[114] It is unclear if selenium supplementation has an effect. [115]

1.2.8. Vaccination

Vaccines have been developed that prevent infection by some carcinogenic viruses. Human papillomavirus vaccine (Gardasil and Cervarix) decrease the risk of developing cervical cancer. The hepatitis B vaccine prevents infection with hepatitis B virus and thus decreases the risk of liver cancer.[116] The administration of human papillomavirus and hepatitis B vaccinations is recommended when resources allow.[117]

1.2.9. Screening

Unlike diagnostic efforts prompted by symptoms and medical signs, cancer screening involves efforts to detect cancer after it has formed, but before any noticeable symptoms appear. This may involve physical examination, blood or urine tests or medical imaging.[118]

Cancer screening is not available for many types of cancers. Even when tests are available, they may not be recommended for everyone. Universal screening or mass screening involves screening everyone.[119] Selective screening identifies people who are at higher risk, such as people with a family history. Several factors are considered to determine whether the benefits of screening outweigh the risks and the costs of screening.[118] These factors include:

- Possible harms from the screening test: for example, X-ray images involve exposure to potentially harmful ionizing radiation
- The likelihood of the test correctly identifying cancer
- The likelihood that cancer is present: Screening is not normally useful for rare cancers.

- Possible harms from follow-up procedures
- Whether suitable treatment is available
- Whether early detection improves treatment outcomes
- Whether the cancer will ever need treatment
- Whether the test is acceptable to the people: If a screening test is too burdensome (for example, extremely painful), then people will refuse to participate.[119]
- Cost

1.3 Diagnosis

Unlike diagnostic efforts prompted by symptoms and medical signs, cancer screening involves efforts to detect cancer after it has formed, but before any noticeable symptoms appear. This may involve physical examination, blood or urine tests or medical imaging.

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- Possible harms from the screening test: for example, X-ray images involve exposure to potentially harmful ionizing radiation
- The likelihood of the test correctly identifying cancer
- The likelihood that cancer is present: Screening is not normally useful for rare cancers.
- Possible harms from follow-up procedures
- Whether suitable treatment is available
- Whether early detection improves treatment outcomes

- Whether the cancer will ever need treatment
- Whether the test is acceptable to the people: If a screening test is too burdensome (for example, extremely painful), then people will refuse to participate

Chemically-derived drugs have been developed and other cancer treatments pre-exist . However, current methods such as chemotherapy have their limitations due to their toxic effects on non-targeted tissues furthering human health problems . Therefore, there is a demand for alternative treatments with naturally-derived anticancer agents with plants being the desired source.

1.4 Prevention

Cancer prevention is defined as active measures to decrease cancer risk. The vast majority of cancer cases are due to environmental risk factors. Many of these environmental factors are controllable lifestyle choices. Thus, cancer is generally preventable. Between 70% and 90% of common cancers are due to environmental factors and therefore potentially preventable.[91]

Greater than 30% of cancer deaths could be prevented by avoiding risk factors including: tobacco, excess weight/obesity, insufficient diet, physical inactivity, alcohol, sexually transmitted infections and air pollution. Not all environmental causes are controllable, such as naturally occurring background radiation and cancers caused through hereditary genetic disorders and thus are not preventable via personal behavior.

1.5. Classification

Cancers are classified by the type of cell that the tumor cells resemble and is therefore presumed to be the origin of the tumor. These types include:

- Carcinoma: Cancers derived from epithelial cells. This group includes many of the most common cancers and include nearly all those in the breast, prostate, lung, pancreas and colon.
- Sarcoma: Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develops from cells originating in mesenchymal cells outside the bone marrow.
- Lymphoma and leukemia: These two classes arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively.[88]
- Germ cell tumor: Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).
- Blastoma: Cancers derived from immature "precursor" cells or embryonic tissue.

Cancers are usually named using -carcinoma, -sarcoma or -blastoma as a suffix, with the Latin or Greek word for the organ or tissue of origin as the root. For example, cancers of the liver parenchyma arising from malignant epithelial cells is called hepatocarcinoma, while a malignancy arising from primitive liver precursor cells is called a hepatoblastoma and a cancer arising from fat cells is called a liposarcoma. For some common cancers, the English organ name is used. For example, the most common type of breast cancer is called ductal carcinoma of the breast. Here, the adjective ductal refers to the appearance of cancer under the microscope, which suggests that it has originated in the milk ducts.

Benign tumors (which are not cancers) are named using -oma as a suffix with the organ name as the root. For example, a benign tumor of smooth muscle cells is called a leiomyoma (the common name of this frequently occurring benign tumor in the uterus is fibroid). Confusingly, some types of cancer use the -noma suffix, examples including melanoma and seminoma.

Some types of cancer are named for the size and shape of the cells under a microscope, such as giant cell carcinoma, spindle cell carcinoma and small-cell carcinoma.

CHAPTER II

2. REVIEW OF LITERATURE

Dong-Jiann Huang et al, (2004)[119] investigated possible antioxidant and antiproliferative activities of the different extracts from sweet potato (Ipomoea batatas [L.] Lam `Tainong 57') organs. DPPH staining, total phenolic compounds and flavonoid content, DPPH radical, reducing power method, FTC method, and cell proliferation were all employed. In the DPPH staining, ethanol extract of vein had the highest radical-scavenging activity when it was diluted to 6.25 mg dry matter/mL. Among all the extracts, the highest amount of total phenolic and flavonoid compounds was found in the ethanol extract of vein. In the DPPH colorimetric method, it was found that ethanol extract of leaf had the highest radical-scavenging activity, followed by water extract of vein. In the reducing power activity assay, it was found that the water extract of leaf had the highest reducing power activity, followed by ethanol extract of vein. Like phenolic compounds, the highest FTC activity was found in the ethanol extract of vein. The antiproliferative activities aof sweet potato were studied in vitro using human lymphoma NB4 cells, and the following results were found: water extract of vein had the highest antiproliferative activity with an $EC_{50}$of 449.6 ± 27.73 µg/mL, followed by water extract of storage root, water extract of leaf, ethanol extract of storage root, and ethanol extract of leaf. Although the ethanol extract of vein showed strong antioxidant activity, it had no antiproliferative activity under the experimental conditions tested.

Prasanth et al (2010)[120] studied invitri cytotoxic and antioxidant properties of ethanolic extract of ipomoea batatus. The extract showed potent cytotoxicactivity in trypan blu

e dye exclusion method using DLA cell lines with EC50 value of 305µg/ml and exhibited a dose dependent decrease in cell count for all the concentrations tested. The antioxidant activity was evaluated by DPPH free radical method. The extract exhibited potent antioxidantactivity with an EC50 of 3 6.5µg/ml.

Wamidh H. Talib et al (2010)[121] Reported Forty four extracts from sixteen plants used traditionally as anticancer agents in vitro for their antiproliferative activity against Hep-2, MCF-7, and Vero cell lines. Plants were fractionated using ethanol, methanol, chloroform, n-hexane, distilled water, and butanol. The antiproliferative activity was measured by MTT assay. TLC was used to identify active fractions. The apoptotic activity of active fractions was determined using TUNEL colorimetric assay. 20 of these extracts demonstrated significant antiproliferative activity against one or more of the cell lines. These extracts were prepared from Ononishirta, Inulaviscosa, Salvia pinardi, Verbascumsinaiticumand Ononissicula. Methanol fractions of Ononishirta(aerial parts) and Inulaviscosa(flowers) were the most active fractions against MCF-7 cells with IC50 of 27.96 and 15.78 µg/ml respectively and they were less toxic against other cell lines. Other extracts showed lower activity against cancer cell lines. TLC analysis showed the presence of flavonoids and terpenoids in active plants while alkaloids were detected in Ononishirta(aerial parts) extracts. Ononishirta(aerial parts) and Inulaviscosa(flowers) extracts exerted their antiproliferative activity by inducing apoptosis in cancer cell lines.

Seow-Mun Hue et al (2011)[122] Evaluated Ipomoea batatas (Sweetpotato) is currently ranked sixth in the total world food production and are planted mainly for their storage roots. The present study was undertaken to evaluate and compare the antioxidant properties of the leaf and carotenoids extract from

the Ipomoea batatas var. Oren leaves. Total flavonoids in the leaf extract was 144.6 ± 40.5 µg/g compared to 114.86 ± 4.35 µg/g catechin equivalent in the carotenoids extract. Total polyphenols in the leaf extracts (3.470 ± 0.024 GAE g/100g DW) was slightly higher compared to carotenoids extract (2.994 ± 0.078 GAE g/100g DW). The carotenoids extract marked a higher radical scavenging capacity with the IC50= 491.86 µg/ml compared to leaf extract (IC50= 545.39 µg/ml). Concentration-dependent reducing activity was observed for both extracts. Thus, the carotenoids extraction process retained most of the antioxidant capacity from the leaves and can be made into potential natural yellow dye with antioxidant property.

Vandana Panda1 et al,(2011)[123] Evaluated Ipomoea batatas (L.) Lam. from the family Convolvulaceae is the world's sixth largest food crop. The tubers of Ipomoea batatas commonly known as sweet potato are consumed as a vegetable globally. The tubers contain high levels of polyphenols such as anthocyanins and phenolic acids and vitamins A, B and C, which impart a potent antioxidant activity that can translate well to show wound healing effects. To check their effects on wound healing, the peels and peel bandage were tested on various injury models in rats in the present study. The methanolic extracts of the peels and peel bandage of Ipomoea batatas tubers (sweet potato) were screened for wound healing by excision and incision wound models on Wistar rats. Three types of gel formulations were prepared, viz., gel containing 3.0% (w/w) peel extract, gel containing 6.0% (w/w) peel extract and gel containing 10% (w/w) peel extract. Betadine (5% w/w povidone iodine cream) was used as a reference standard. In the incision wound model, Tensile strength of the skin was measured. Epithelization time, wound contraction, hydroxyproline content of the scab, and ascorbic acid and malondialdehyde content of the plasma were determined in the excision wound model. Results: In the incision

wound model, high tensile strength of the wounded skin was observed in animals treated with the peel extract gels and the peel bandage when compared with wounded control animals. The increase in tensile strength indicates the promotion of collagen fibers and that the disrupted wound surfaces are being firmly knit by collagen. In the excision wound model, significant wound closure was observed on the 4 th day in rats treated with all three gel Functional Foods in Health and Disease 2011; 10:403-415 Page 404 of 415 formulations when compared with the wounded control rats. A significant increase in hydroxyproline and ascorbic acid content in the gel-treated animals and a significant decrease in malondialdehyde content in the animals treated with gel as well as peel bandage was observed when compared with the wounded control animals. It may be concluded that the peels of Ipomoea batatas tubers possess a potent wound healing activity, which may be due to an underlying antioxidant mechanism.

Vandana Panda et al, (2012)[124] Conducted studies on peptic ulcers occur in that part of the gastrointestinal tract which is exposed to gastric acid and pepsin, i.e., the stomach and duodenum. Gastric and duodenal ulcers are common pathologies that may be induced by a variety of factors such as stress, smoking and noxious agents including non-steroidal anti-inflammatory drugs. Ipomoea batatas tubers (sweet potato) contain ample amounts of antioxidants. It has been proven already by many scientific studies that antioxidants have ulcer healing properties. In reference to this, we tried assessing the ulcer healing effect of Ipomoea batatas tubers. The anti-ulcer activity of the tubers of Ipomoea batatas (sweet potato) was studied in cold stress and aspirin-induced gastric ulcers in Wistar rats. Methanolic extracts of Ipomoea batatas tubers (TE) at two doses, viz., 400 and 800 mg /kg were evaluated in cold stress and aspirin-induced gastric ulcer models using cimetidine

and omeprazole respectively as standards. The standard drugs and the test drugs were administered orally for 7 days in the cold stress model and for 1 day in the aspirin-induced gastric ulcer model. Gastroprotective potential, status of the antioxidant enzymes {superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase(GR)} along with GSH, and lipid peroxidation were studied in both models. Results: The results of the present study showed that TE possessed gastroprotective activity as evidenced by its significant inhibition of mean ulcer score and ulcer index and a marked increase in GSH, SOD, CAT, GPx, and GR levels and reduction in lipid peroxidation in a dose dependant manner. Conclusion: The present experimental findings suggest that tubers of Ipomoea batatas may be useful for treating peptic ulcers.

Yuzhijiao et al (2012)[125] The radical scavenging effects by α,α-diphenyl-β-picrylhydrazyl (DPPH) and superoxide anions of anthocyanin extract from purple sweet potato were investigated. The antioxidation experiments showed that the reducing power of the anthocyanin extract reduced 0.572 at 0.5 mg/ml, while those of Lascorbic acid (L-AA) and butylatedhydroxytoluene (BHT) reduced 0.460 and 0.121, respectively. They also displayed potent antioxidant effects against the DPPH radical and superoxide anions radical, showing the IC50 values of 6.94 and 3.68 μg/ml, respectively. Moreover, this anthocyanin extract also could significantly inhibit the formation of lipid peroxidation compound. Sixteen kinds of anthocyanins in purple sweet potato were detected by high-performance liquid chromatography with diode-array detection (HPLC-DAD), and most of the anthocyanins were acylated.

Marilena Meira et al (2012)[126] ,Approximately 600-700 species of *Ipomoea*, Convolvulaceae, are found throughout tropical and

subtropical regions of the world. Several of those species have been used as ornamental plants, food, medicines or in religious ritual. The present work reviews the traditional uses, chemistry and biological activities of *Ipomoea* species and illustrates the potential of the genus as a source of therapeutic agents. These species are used in different parts of the world for the treatment of several diseases, such as, diabetes, hypertension, dysentery, constipation, fatigue, arthritis rheumatism, hydrocephaly, meningitis, kidney ailments and inflammations.Some of these species showed antimicrobial, analgesic, spasmolitic, spasmogenic, hypoglycemic, hypotensive, anticoagulant, anti-infl ammatory, psychotomimetic and anticancer activities. Alkaloids, phenolics compounds and glycolipids are the most common biologically active constituents from these plant extracts.

Adsull et al (2012)[127] Microbial diseases remain a major challenge for modern science even today. Natural products are used as traditional medicines from ancient times. They are having a great importance in Ayurveda. One of the medicinal plant species is Ipomoea carnea belongs to convolvulaceae family and fistulosa sub-family. Antimicrobial activity was tested against n-hexane, ethyl acetate, acetone, ethanol and acetone fraction of acetone extract. The investigation is carried out against various gram positive and gram negative bacterial strains (Escherichia coli ATCC – 11246; Staphylococcus aureus ATCC – 6538 P; Salmonella typhimurium ATCC – 23564; Pseudomonas aeruginosa ATCC – 27853; Proteus vulgaris ATCC – 13315; Bacillus cereus ATCC – 11778) . Disc diffusion method is employed for the detection of antimicrobial activity. Streptomycin was used as standard. The crude acetone extract (3) exhibits activity against Proteus vulgaris and Salmonella typhimurium, while the crude ethanol extract (4) ellucidates antimicrobial activity against Pseudomonous auroginosa

RajuAsirvatham et al (2013)[128] Conducted in vitro studies was performed to examine the antioxidant and anticancer activities of ethanol and aqueous extracts of DroseraindicaL. Methods: Different concentrations (5 – 640mcg/ml) of the ethanol (EEDI) and aqueous (AEDI) extracts of D.indicaL were used in various antioxidant assay methods such as hydroxyl radicals, DPPH, super oxide radical scavenging activity, chelating ability of ferrous ion, nitric oxide radical inhibition, ABTS and reducing power. Ascorbic acid (AA) was used as the standard antioxidant for the free radical scavenging assays. Dalton's Ascitic Lymphoma (DAL) and Ehrlich Ascitic Carcinoma (EAC) cell lines were used as the in vitro cancer models for the tryphan blue dye and LDH leakage assays, where 5 to 250mcg /ml of both EEDI and AEDI were tested. Results: EEDI showed antioxidant activities with the minimum IC50 values of 34.8±0.43 mcg/ml in scavenging of hydroxyl radical and moreover AEDI showed minimum IC50 values of 94.51±0.84 mcg/ml in Fe2+chelating assay. EEDI on the reducing power assay and ABTS showed higher IC50 than standard AA. IC50 values of AEDI on Fe2+ chelating assay and super oxide radical assay was lesser than IC50 value of AA. Both extracts at 250mcg/ml dose showed remarkable increase in the percentage of dead cancer cells (90% by EEDI and 86% by AEDI in DAL model and 89% by EEDI and 80% by AEDI in EAC model).

Eleazu et al(2013)[129] Evaluated The physicochemical composition, functional properties, inhibitory actions and energy value of the flour of a cream fleshed sweetpotato variety (TIS/87/0087) that is high yielding and commercially sold in South Eastern Nigeria were investigated using standard techniques. The flour was observed to have good functional properties with a pH of 5.32±0.01, high percentage moisture content, indicative of poor shelf life characteristics and high chances of being attacked by microbes, low percentage dry

matter, lipid, crude fibre and ash contents but a promising source of starch (20.78±0.02%), carotene (5.0±0.04 µg/g), protein (2.67±0.59%), carbohydrate (40.77±3.05%), energy (179.61±20.97 kcal/100 g), polyphenols, in addition to containing significant quantities of reducing sugar (1.58±0.53%). In addition, the methanolic extract of the flour possessed higher scavenging activities on 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical than standard quercetin. Results show that this sweet potato variety has potentials of biological properties and could have wide utility in food, alcohol and sugar industries. In addition, it could serve as a promising source of protein and its consumption could be utilized in the management of diseases that implicate free radicals. Finally, it could also be useful as a drug binder and disintegrant in pharmaceutical industries.

Zainal Baharum et.al (2014)[130] The aims of this study were to determine the antioxidant and antiproliferative activity of the following *Theobroma cacao* plant part methanolic extracts: leaf, bark, husk, fermented and unfermented shell, pith, root, and cherelle. Antioxidant activity was determined using 2,2-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid-reactive substances (TBARS), and Folin-Ciocalteu assays; diphenyltetrazolium (MTT) assay was used to determine antiproliferative activity. The root extract had the highest antioxidant activity; its median effective dose (EC_{50}) was 358.3 ± 7.0 µg/mL and total phenolic content was 22.0 ± 1.1 g GAE/100 g extract as compared to the other methanolic plant part extracts. Only the cherelle extract demonstrated 10.4% ± 1.1% inhibition activity in the lipid peroxidation assay. The MTT assay revealed that the leaf extract had the highest antiproliferative activity against MCF-7 cells [median inhibitory concentration (IC_{50}) = 41.4 ± 3.3 µg/mL]. Given the overall high IC_{50} for the normal liver cell line WRL-68, this study indicates that *T. cacao* methanolic extracts have a cytotoxic effect in cancer cells, but not in normal cells. Planned

future investigations will involve the purification, identification, determination of the mechanisms of action, and molecular assay of *T. cacao* plant extracts.

Marcelia Sugata et.al (2015)[131] Purple-fleshed sweet potato (PFSP) Tainung 73 possesses high amount of antioxidative compounds, such as phenolics, flavonoid, and anthocyanin. The major anthocyanin is cyanidin or/and peonidin and their acylated derivatives. Study on the possible properties of PFSP extracts showed that these extracts had potential anti-inflammatory and anticancer activities. Anthocyanin-rich extracts of PFSP TNG 73 could suppress the production of nitric oxide (NO) and some proinflammatory cytokines, such as NFκ-β, TNF-α, and IL-6, in LPS-induced macrophage cell. Nevertheless, these extracts showed no cytotoxicity effect on macrophage cells. On the other hand, these extracts could inhibit the growth of some cancer cell lines, such as human breast cancer (MCF-7), gastric cancer (SNU-1), and colon adenocarcinoma (WiDr), in concentration- and time-dependent manner. After further investigation on molecular mechanism, PFSP TNG 73 extracts demonstrated the ability to induce apoptosis in MFC-7 cancer cell line through extrinsic and intrinsic pathways. Thus, PFSP TNG 73 can be used for future application of drugs, nutritional food, and health supplement.

Milind parle et al (2015)[132] Sweet potato is an extremely versatile and delicious vegetable that posses high nutritional value. Sweet potato has been grown in tropical and subtropical regions throughout the world, since ancient times. From the times immemorial, the whole sweet potato plant including leaves, stem, and tuberous root is used as traditional medicine. Nowadays, Sweet potato is preferred over other vegetables due to its multifaceted medicinal properties. The medicinal properties of sweet potato include anti-cancer, anti-inflammatory, anti-

diabetic, anti-oxidant, anti-bacterial, anti-fungal, anti-viral, anti-ulcer, hepatoprotective, wound healing and immunomodulatory activities. Sweet potatoes contain magnesium, a crucial mineral, which promotes relaxation, calmness and nerve health. Overall objective of this review article is to give a brief knowledge about the nutritional value, health benefits, phytochemical composition, pharmacological actions and medicinal properties of sweet potato. Sweet potato holds first rank (super food) in nutrition among vegetables.

HuaJi et al (2015)[133] In this study, we selected four different color fleshed sweet potatoes, purple- (Jizi 01), red- (Xinong 431), yellow- (Beijing 553) and white- (Shangshu 19) fleshed cultivars as test materials, analyzed nutrient composition, dietary fiber content, anthocyanins quantification, and total phenolics content, and also measured their total antioxidant activity in four different types of sweet potato. In view of differences in flesh color, the nutrient contents of different cultivars appeared to be significantly different. Starch contents of Beijing 553 and Shangshu 19 were higher, but fat contents were lower than others. Protein content of Shangshu 19 was the highest followed by Jizi 01 and Xinong 431. In addition, our analysis results confirmed that purple fleshed sweet potato possesses much higher anthocyanins content than others, even up to 6.23 mg/g dry matter. Also, dietary fiber, total phenolics content, and total antioxidant capacity of Jizi 01 were significantly higher.

Sucharitha et al (2016)[134] A diuretic is any substance that promotes the production of urine. This includes forced diuresis. There are several categories of diuretics. All diuretics increase the excretion of water from bodies, although each class does so in a distinct way. Alternatively, an ant diuretic such as vasopressin, or ant diuretic hormone, is an agent or drug which

reduces the excretion of water in urine. In medicine, diuretics are used to treat heart failure, liver cirrhosis, hypertension, influenza, water poisoning, and certain kidney diseases. Some diuretics, such as acetazolamide, help to make the urine more alkaline and are helpful in increasing excretion of substances such as aspirin in cases of overdose or poisoning. Diuretics are often abused by those with eating disorders, especially bulimics, in attempts to lose weight. The antihypertensive actions of some diuretics (thiazides and loop diuretics in particular) are independent of their diuretic effect. That is, the reduction in blood pressure is not due to decreased blood volume resulting from increased urine production, but occurs through other mechanisms and at lower doses than that required to produce diuresis. Indapamide was specifically designed with this in mind, and has a larger therapeutic window for hypertension (without pronounced diuresis) than most other diuretics. The main objective of the present research work is to isolate the bioactive molecules and evaluate the diuretic activity of aqueous extract of Ipomoea batatasthe phytochemical analysis of aqueous extract of Ipomoea batatas root showed the presence of various phytochemical constituents such as flavonoids, carbohydrates, tannins, phenol. The effect of aqueous extract of root of Ipomoea batatas on rats with reference to biochemical changes in serum. The group-II (Standard Hydrochlorothiazide 10 ml/kg, p. o) animals showed significant ($P<0.01$) increase in total urine volume ml/100 gm/hr (10.44 ml). Whereas animals received AEIB significantly ($P<0.01$) increase in total urine volume ml/100 gm/hr (4.44 and 8.06 ml) and significantly ($P<0.05$) increased total 200 & 400 mg/kg doses respectively. The phytochemical studies revealed the presence of Carbohydrate, flavonoids, Tannins in the AEIB these may be responsible for its pharmacological activities.

CHAPTER III

.3. AIM AND OBJECTIVE

Cancer is one of the major problem in worldwide due to life style, usage of cancer medications, tobacco usage, lack of proper medication, and undefined drug targets. The treatment of cancer after so many years of research and experience is still unsatisfactory due to certain special characteristics of the cancer cell like capacity for uncontrolled proliferation, invasiveness, metastasis and dedifferentiation. Such dedifferentiated cancer cells can multiply faster when compared to well differentiated cancer cells. Several modern drugs are available in the market do not fulfill the requirements and also with many side effects. So the priority is drugs which minimize the side effects and also required chemical entity for the treatment of cancer with specific action. Several literatures indicated traditional herbs possessing lesser side effects compared to synthetic drugs. The herbal formulations which developed from ayurveda, traditional system of Indian medicine and its additional system of medicine, has been found to have anticancer activity. *Ipomoea batatus* is a traditionally used herb which posses anticancer activity but no scientific validation.. Some scientific studies show that leaf and root extract of *ipomoea batatus* posses anticancer activity. But there was no literature review about anticancer property of the stem available. So the present study is an attempt to develop plant based anticancer drug which will be lesser side effect.

CHAPTER IV

4. PLAN OF WORK

I. literature Surevey

II. Extraction of dried stem of the *Ipomoea batatus* by using Soxhlet Apparatus.

III. Preliminary phytochemical screening of the ethanolic extract of the plant using specific chemical test.

IV. Perform *invitro* Anti cancer activity will carried out by effect of ethanolic extract of the stem of *ipomoea batatus* on MTT assay.

V. Selection of fraction with higher activity.

VI. The *Invivo* anticancer activity of ethanolic extract of stem of *Ipomoea batatus* by using DLA Method in mice.

VII. Estimating the effect of ethanolic extract of stem of ipomoea batatus on

- Mean survival time
- Hemoglobin Count
- RBC Count
- WBC Count

VIII. Confirmining the anticancer activity of active constituents from literarture.

IX. Conclusion of the work.

CHAPTER V

5. PLANT PROFILE

5.1. Plant profile

Kingdom : *plantae*

Subkingdom : *Tracheobionta*

Super division: *Spermatophyte*

Division : *Sagnoliophyta*

Class : *Magnoliopsida*

Sub class : *Asteridae*

Order : *Solanales*

Family : *Convolvulaceae*

Genus : *Ipomoea L.*

Species : *I. batatas* (L.) *LAM*

Synonyms :

Kannada : *Genasu.*

Hindi : *Shakarkand / Ratalu.*

Telugu : *Chilakada dumpa*.

Marathi : *Ratala.*

Bengali : *MishtiAlu.*

Malayalam : *Mathura Kizhangu.*

Scientific names

batatasedulisChoisy (Kamote (all dialects)

Convolvulus batatas Linn (Lapin (if.)

Convolvulus edulisChoisy (Pangg-bagun (Sul.)

Ipomoea batatas (L.) (Sweet potato (Engl.),

(Yam (Engl.),

5.2. Geographical distribution:

The plant is commonly seen growing in all parts of India, Asian and some other European and American countries(9).

5.3. Chemical constituents of leaf:

Sweetpotato roots and tops possess a variety of chemical compounds relevant to human health. About 80 to 90 % of sweetpotato dry matter is made up of carbohydrates, consisting mainly of starch (60-70%) and sugars with lesser amounts of pectins, hemicelluloses and cellulose. Sweet-potato also contains

protein (0.46%-2.93%), dietary fiber (0.49%-4.71%), lipid (0.06%-0.48%) and ash (0.31%-1.06%).

It contains essential mineral nutrients such as Ca(117mg), (0.56mg),vit E(0.56mg), Fe(1.8mg)/100mg, S, Cu, Zn, P, Mg, Na, K, Mn, Al and B.Sweetpotato is also an important source of vitamin A, thiamin, riboflavin, niacin, ascorbic acid,β-carotene and many other functional compounds.

Sweetpotato leaves are an excellent source flavonoids with antioxidative poly-phenols, with 6 polyphenolic compounds ,15 anthocyanins and phenolic acids such as caffeic, monocaffeoylquinic (chloro-genic), dicaffeoylquinic and tricaffeoylquinic acids, and are superior in this regard to other commercial vegetables.

The another major constituent flavonoids is proanthocyaniins and two or more flavan-3-ol such as catechin, epicatechin or gallocatechin. Catechin contains 2 benzene rings to be the powerful scavenger(8).

5.4. Uses Of Ipomoea Batatas

1 :The young leaves and shoots are sometimes eaten as greens.

2 : All parts of the plant are used for animal fodder.

3 : Industrial alcolol production.

4: Sweetpotato leaves used as a vegetable, a tea, in noodles, in breads, in confec-tioneries and as a nutritional supplement.

Reported activities of Ipomoea batatas:

1: Makoto yoshimoto and shoji yahara evaluated the anti-mutagenic activity of the caffeoylquinic acid derivatives in Ipomoea batatas leaf.

2: poly phenolics have attracted special attention due to their use in oxidative stress which may cause cancer, aging.

3: Mukesh Nandave, SK Ojha and DS Arya reported that flavonoids show anti-thrombotic, anti-ischemic, anti-arrythmic and cardioprotective activity by free radical scavenging mechanism.

4: The leaf extracts also shows anti-bacterial activity, ultraviolet protection effect, anti-inflammation and promotion in bowel movement.

Folkloricuse:
-Crushed leaves applied to boils and acne.
-Boiled roots used for diarrhea.

"Ipomoea" comes from the Greek words ipos, which means “bind weed” and homoios which means "resembling". When this is put together to form "Ipomoea" the direct translation is "resembling bindweed". This name makes sense because the sweet potato has a twining habit, much like the bindweed. The species name "batatas" was originally the Taino name for sweet potato.

Ipomoea batatas is a perennial climber growing up to 3 meters at a fast rate. This is one of those rare plants that are grown as a tasty vegetable and a pretty ornamental. Sweet potato vine is a vigorous, herbaceous, trailing vine, is native to Central America and the Pacific Islands. It offers sweet edible tubers and leaves and attractive foliage.

The leaves are heart-shaped or deeply lobed the colors vary from chartreuse, to bronze or purple and can be variegated. The funnel-shaped flowers can be pale rosy purple or white. They appear when days are long and growing conditions favorable.

CHAPTER VI

6. MATERIALS AND METHODS

6.1. Collection of plant

The stem of Ipomoea batatus were obtained from Department of Horticulture, Malappuram and it was authenticated by Dr.Raghu.A.V Kerala Forest Research Institute Peechi, Trissur.

6.2. Reagents and Requirements for preliminary extraction procedure

1. Reflux apparatus.
2.Methanol (Merk Germany)
3.Dried stem of Ipomoea batatus
4.Glass ware apparatus
5.Dessicator

6.3. Extraction of plant material

6.3.1. Extraction 1

About 100 g of powdered sample was weighed and extracted with 500 ml of methanolby reflux method at 24 hours. After the reflux, extract was collected dried under reduced pressure, which was stored in desiccators. Complete moisture was removed from the extract and yield was calculated by using the following formula

Percentage yield = (B/A) X 100

Where

A = Weight of powder

B = Weight of extract

6.3.2. Extraction 2

About 100 g of dried stem was taken, powdered by a grinder, weighed and extracted with 500ml of chloroform by reflux method at 24 hours. After the reflux, extract was collected dried under reduced pressure which was stored in desiccators in order to remove complete moisture from the extract and yield was calculated by using the above formula.

6.3.3. Extraction 3

About 100g of powdered sample was weighed and extracted with 500ml of ethanol by reflux method at 24 hours. After the reflux, the extract was collected dried under reduced pressure, which was stored in desiccators until complete moisture was removed from the extract and yield was calculated by using the above formula.

6.4. Preliminary phytochemical screening of the plant

The ethanol extract of the plant was used for testing preliminary phytochemical screening in order to detect major groups.

6.4.1. Test for carbohydrates

a. **Molich's test:** Dissolve small quantity of ethanolic extract of Ipomoea batatusseperatlyin a 4ml distilled water and filtered. The filterate was subjected to Molisch's test.
b. **Fehling's test:** Dissolve a small portion of extract in water and treat with Fehling's solution.
c. **Phenols test:** The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapour.

6.4.2. Test for flavonoids

a. **Shinoda test:** To the 2 to3 ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCL was added.
b. **Lead acetate test:** To the 5ml extract 1 ml of lead acetate solution was added.

6.4.3. Test for tannins

a. **Braemer's test**: to the 2 to 2 ml extract, 10% alcoholic ferric chloride solution was added.

6.4.4. Test for steroid/terpinoid

a. **Libbermann-Burchardt test**: To 1 ml of extract, 1 ml of chloroform, 2 to 3 ml of acetic anhydride and 1 to 2 drops of concentrated sulphuric acid are added.

6.4.5. Test for alkaloids

a. **Draggendrofs test**: A drop of extract was spottec on small pieces of precoated TLC plate and the plate was sprayed with modified Draggendrofs reagent
b. **Hager's test**: The extract was treated with few ml of Hager's reagent.
c. **Wagner's test**: The extract was treated with few ml of Wagner's reagent.

6.4.6. Test for Glycosides

a. **Legal's test**: Dissolve the extract in 2 ml pyridine, add sodium nitroprusside solution 2 ml, and made alkaline with sodium hydroxide solution.

6.4.7. Test for Saponins

a. **Foam test**: 1 ml of extract was diluted with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

6.4.8. Test for Amino acids

a. **Ninhydrin test**: Dissolve a small quantity of extract in few ml of water and added 1ml of ninhydrin reagent.

6.4.9. Test for anthraquinones

a. **Borntrager's test:** About 50mg of powder extract was treated with 10% of ferric chloride solution and 1 ml of concentrated HCL. The extract was cooled, filtered, and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

6.4.10. Test for fixed oils and fats

a. Press small quantity of the petroilum ether extract between two filter paper.

The results of the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response to the test is average it can be noted as ++ which indicates the presence in moderate quantity.
- If the response is very small note it as + indicating the presence of only in traces the response is very small
- If no response is then negative.

6.5. Pharmacological Screening

6.5.1. Invitro Anticancer Activity

6.5.2. Mtt Assay: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 16;65(1-2):55-63

6.5.3. Tumour Cells

Tumor cells Ehrlich Ascites Carcinoma (EAC) cells were obtained from Amala cancer research center, Trissur, Kerala, India. The cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation. EAC cells aspirated from the peritoneal cavity of mice were washed with saline and given intraperitoneally to develop ascetic tumor.

6.5.2. Mtt Assay (Helacell Lines)

1. For adherent cells, remove the medium and replace it with 100 µL of fresh culture medium. For non-adherent cells, centrifuge the microplate, pellet the cells, carefully remove as much medium as possible and replace it with 100 µL of fresh medium.
2. Add 10 µL of the 12 mM MTT stock solution (prepared in step 1.1) to each well. Include a negative control of 10 µL of the MTT stock solution added to 100 µL of medium alone.
3. Incubate at 37°C for 4 hours. At high cell densities (>100,000 cells per well) the incubation time can be shortened to 2 hours.
4. Add 100 µL of the SDS-HCl solution (prepared in step 1.2) to each well and mix thoroughly using the pipette.

5. Incubate the microplate at 37°C for 4– hours in a humidified chamber. Longer incubations will decrease the sensitivity of the assay.
6. Mix each sample again using a pipette and read absorbance at 570 nm.

6.5.4. Animals

Male Swiss albino mice weighing 25- 30g were used in the present study. All rats were kept at room temperature of 22-25ºC in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions. All animal procedures were performed after approval from the institutional ethics committee. The experimental protocol has been approved by institutional animal ethics committee, The Karpagam College of Pharmacy, Coimbatore

6.5.5. Acute toxicity study

Ethanolic extract of Ipomoea batatus stem were studied for acute toxicity at doses of 5mg/kg, 50mg/kg, 300mg/kg, 500mg/kg and 2000mg/kg. As per OECD 420 guideline dose of 2000mg/kg showed the toxic symptoms, so according to OECD guideline 420, it is considered as a LD50 cutoff value. Doses selected for pharmacological studies by fixed dose methods are 250mg/kg and 500mg/kg8.

6.6.Invivo Anticancer Activity

The Swiss albino mice (20-25 g) were divided into five groups (n=12). Except, Group-I all the animals in DLA group were being injected with DLA cells (2×106 cells/mouse, i.p). This was marked as day “0”. Group-I was served as normal DLAcontrol and group-II was served as standard group. After 24h, DLA transplanted group-III, IV and V were being injected (100, 200 and 400mg/kg b.w. i.p.) once daily for 12 consecutive days. Group II received standard drug 5-Flurouracil (20 mg/kg i.p) for 12 consecutive days. After administrations of last dose 6 mice from each group were kept fasting for 18h and blood was collected by direct cardiac puncture for the estimation of haematological determination. Rest of animals in each groups were kept alive with food and water ad libitum to check the percentage increase in life span of the tumor host and also to determine the mean survival time (MST).Antitumor activity of MeIB extract was assessed by observation of changes with respect to the following parameters

6.6.1. Antitumour Parameters

Percentage increase life span The effect of extract on tumor growth was monitored by recording the mortality daily for a period of 6 weeks and percentage increase in average life span was calculated. % ILS = {(life span of treated group/ life span of controlled group)-1} X 100

6.6.2. Body weight analysis

Body weights of the experimental mice were recorded both in the treated and control groups at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period and calculated on 15th day.

6.6.3. Changes in food intake

Feed consumed by 6 animal/cage/week = Total quantity of feed offered during that week (gm) – Feed left over on last day of week (gm). Feed consumed by individual animal/week = Feed consumed by 6 animal per cage per week/6. Feed consumed by individual animal/day =Feed consumed by individual animal per week/7.

6.6.4. Determination of tumor volume and weight

The mice were dissected and the ascetic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and weight immediately.

6.6.5. Determination of Hematological parameters

Blood was drawn from each mouse by the retro orbital plexus method and the white blood cells (WBC), red blood cells (RBC), hemoglobin and protein were determined. Serum preparation of the sample blood use to evaluated the biochemical parameters.

6.7. Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison tests. P value < 0.001 was considered as highly significant and < 0.05 were considered significant when compared to control.

CHAPTER VII

7. RESULTS AND DISCUSSION

7.1 Soxhlet Extraction of *Ipomoea Batatus* Stem

The percentage yield of various extracts viz. petroleum methanol, chloroform and ethanol was 23.98%, 35.83%, 20.46%w/w respectively

Table No:1-The percentage yield of stem of Ipomoea batatus stem extracts

Plant	**Part used**	**Method of Extraction**	**Solvents**	**Percentage Yield (%W/W)**
Ipomoea batatus	Dried stem	Continuous Hot percolation by Soxhlet apparatus	Chloroform	23.98%
			Ethanol	35.83%
			Methanol	20.46%

Figure No: 3 Soxhlet Apparatus

.

7.2 Preliminary phytochemical screening of *Ipomoea batatus* stem extract

Table No:2 Preliminary phytochemical screening of *Ipomoea batatus* stem extract

Class of compounds	**Tests performed**	**Results**
Carbohydrates	Molisch's test Fehling's test	**Negetive**
Phenols	Phosphomolybdic acid test	**Positive**
Flavonoids	Shinoda test Lead acetate test	**Positive**
Tannins	Braemer's test	**Negetive**
Alkaloids	Wagner's Mayer's Draggendrof's test	**Positive**
Glycosides	Legal's test Brontranger's test	**Positive**
Saponins	Foam test	**Positive**
Sterols	Salkowski's test	**Negetive**
Aminoacids	Ninhydrin test	**Negetive**
Terpenoids	LibermannBurchardt test	**Negetive**

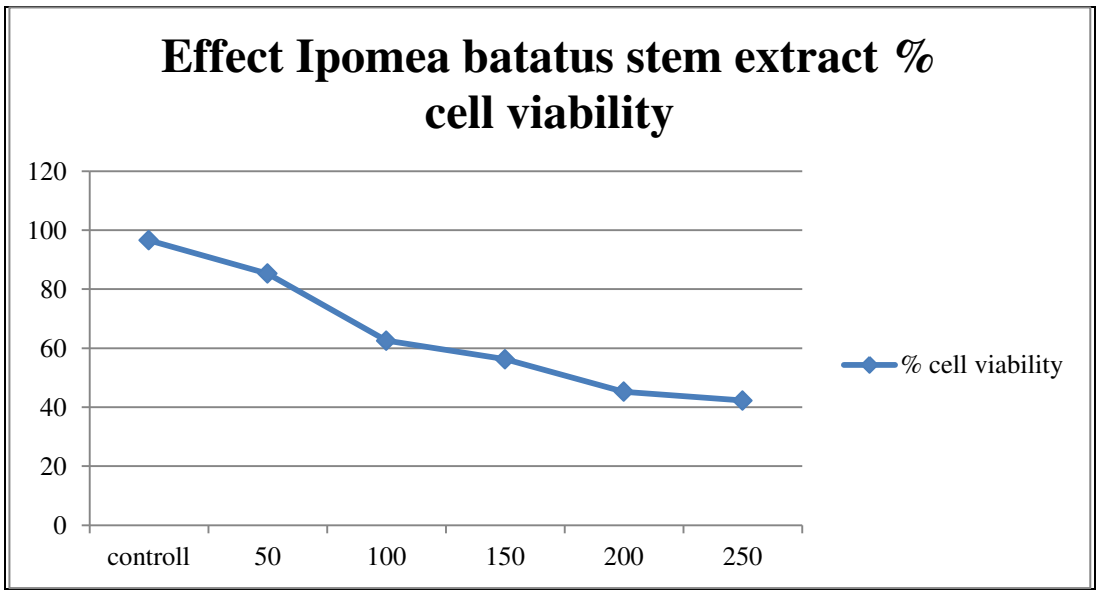
7.3 *Invitro* Anticancr Activity

The percentage cell viability is reduced with increase in dose of the plant extract.

Table No: 3 Concentration vs Percentage viability

Sample Concentration (µg/ml)	MTT assay –HeLa % viability
Control	96.58±0.25
50	85.26±0.21
100	62.54±0.34
150	56.23±0.38
200	45.23±0.36
250	42.25±0.40

Figure No.3 Effect *Ipomoea batatus* stem extract on percentage cell viability



7.4 Acute Oral Toxicity Studies (OECD 2001)

After administrating the drug we checked the animals for 15 days. The parameters checked includes Mortality rate, Mucous membrane, salivation hair falling etc. all the parameters were found to be normal.

Table No.4 - Common Parameters

Parameters	DLA controll	Standard (cyclophosphamide)	Extract 100mg/kg	Extract 200mg/kg	Extract 400mg/kg
Skin and fur	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal
Behavioural patterns	Normal	Normal	Normal	Normal	Rapid heart beat
COMA	Normal	Normal	Normal	Normal	Normal

Mortality rate is found to be zero percentage. None of the animals died in between the experiment. It shows the extract posses lesser side effect and can be used safely.

Table No: 5 - Mortality rate

Group	Dose (mg/kg)	No.of animals	Dose difference (a)	Mortality Rate (b)
1.	5	6	0	Zero Percentage mortality rate
2.	50	6	45	
3.	300	6	250	
4.	2000	6	1750	

LD_{50} = higher dose – Σ (a x b)/ n Where n = No. of animals in each group

LD_{50} = 2000– 0 = 2000 mg / kg

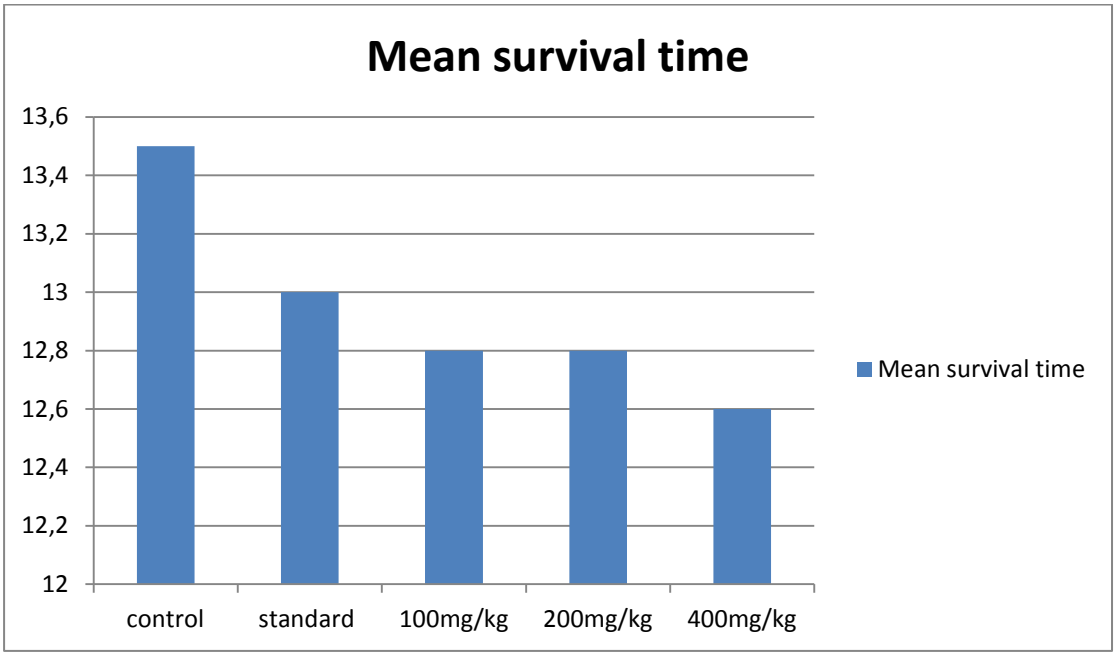
ED_{50} = LD_{50} / 10 = 2000 / 10 = 200mg / kg

7.5 Invivo Anticancer Studies

Table No:7 Effect Of Ipomoea Batatus Stem Extract On Mean Survival Time

PARAMETERS	CONTROL	STANDARD (20mg/kg)	EXTRACT (100mg/kg)	EXTRACT (200mg/kg)	EXTRACT (400mg/kg)
Mean survival time (days)	14.5±0.14	14±0.14***	13.8±0.28*	13.1±0.42*	12.9±0.28***

Figure No: 7- Effect Of Ipomoea Batatus Stem Extract On Mean Survival Time



7.6 Effect Of *Ipomoea Batatus* Stem Extract On Hemoglobin

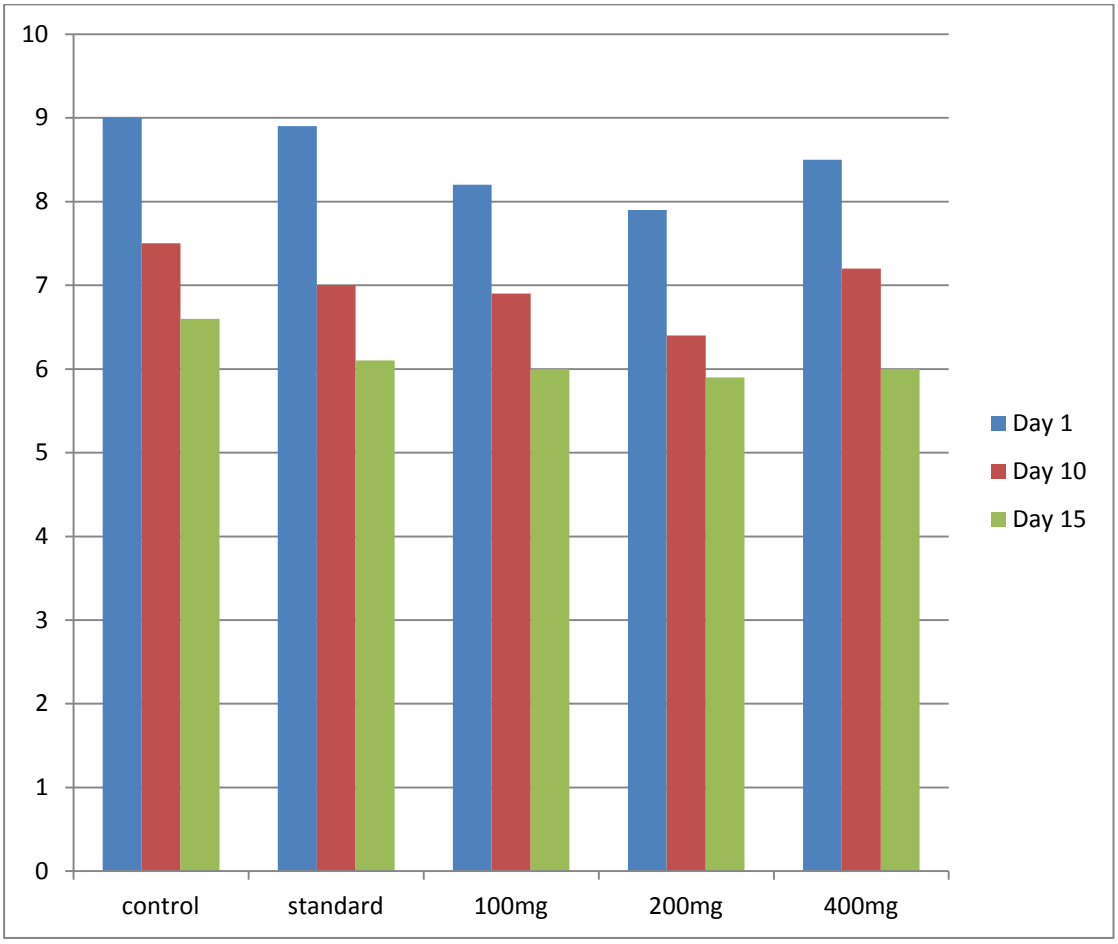
The *invivo* immunomodulatory activity results have been expressed that the ethanolic extract of stem of ipomoea batatus shown anticancer activity action when compared to control and positive control which is used in dose levels at100, 200 and 400mg/kg have dose dependend anti cancer activity due to constantly decreased Hemoglobin. From the figure the anti cancer activity conducted on ethanolic extract of fruits of ipomoea batatus on Hemoglobin count in mice showed the different drug concentration is less activity as compared to standard drug. After 15 days of analysis the Hemoglobin count is less in different drug concentration as compared to control drug.

Table No.8 Effect Of *Ipomoea Batatus* Stem Extract On Hemoglobin

Group N=6	**Treatment**	**Dose (mg/kg body weight)**	**Hemoglobin count**		
			Day1	**Day 10**	**Day 15**
Group I	controll	0.6%	9.0±0.13	7.5±0.15	6.6±0.16
Group II	cyclophosphamide	30	8.9±.018	7.0±0.19	6.1±0.17
Group III	Ethanolic extract	100	8.2±0.22	6.9±0.21	6.0±0.22
Group IV	Ethanolic extract	200	7.9±0.26	6.4±0.25	5.9±0.26
Group V	Ethanolic extract	400	8.5±0.29	7.2±0.30	6.0±0.31

All values are expressed as mean ± SEM evaluated by ANOVA followed by Dunnet's t test

Figure No: 9 Effect Of Ipomoea Batatus Stem Extract On Hemoglobin Count



Drug concentration vs Hb concentration

7.7 Effect Of *Ipomoea Batatus* Stem Extract On RBC

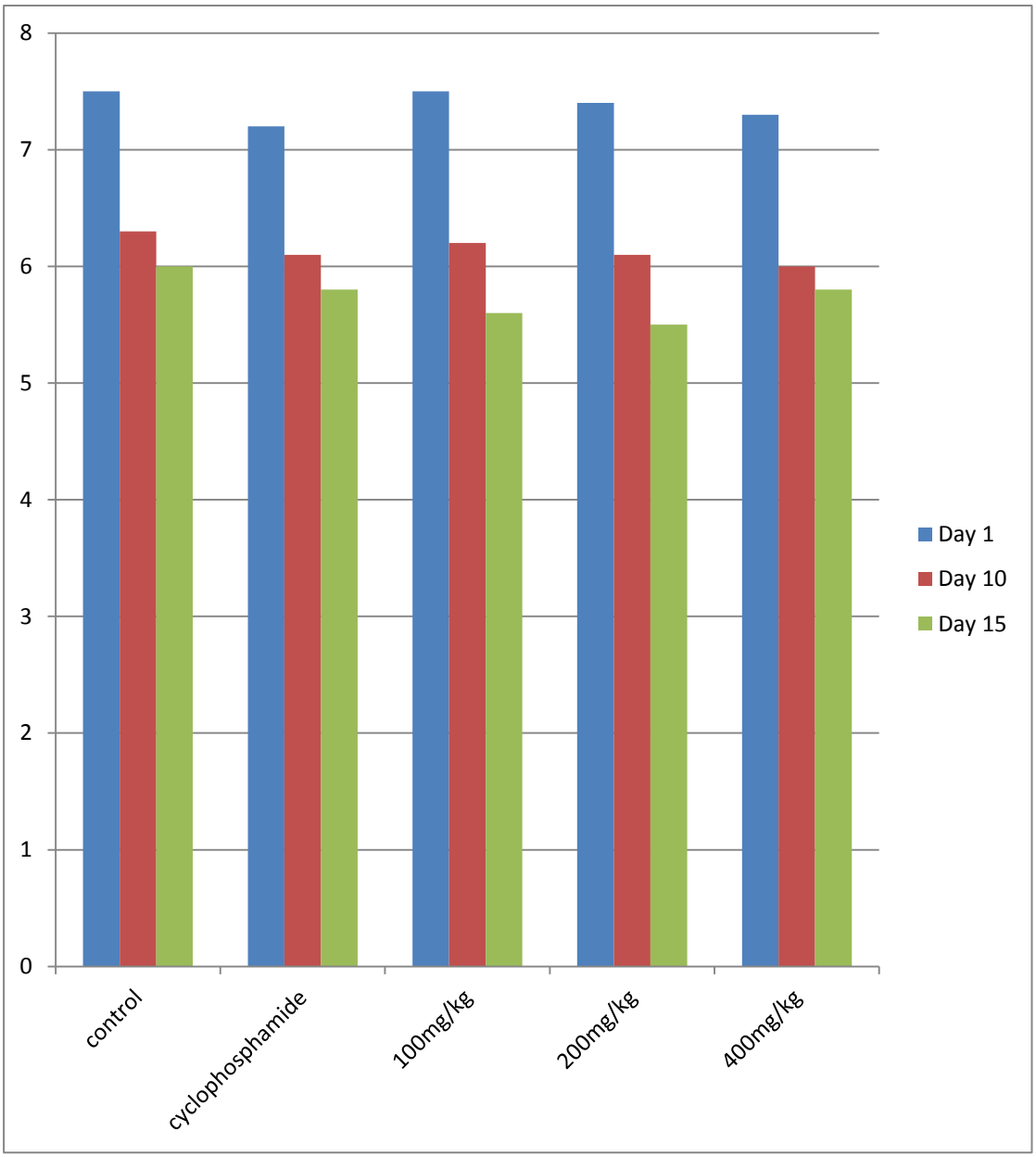
The *invivo* anticancer activity results have been expressed that the ethanolic extract of stem of *ipomoea batatus* shown anticancer activity, when compared to control and positive control which used dose levels at 100,200 and 400mg/kg have dose dependent anticancer activity due to constantly decreased RBC cells

Table No:10 Effect Of *Ipomoea Batatus* Stem Extract On RBC Count

Group (N=6)	Treatment	Dose (mg/kg Body weight)	RBC COUNT (x 10^6/µL)		
			Day 1	Day 10	Day 15
Group I	Control	6%	7.5±0.32*	6.3±0.35	6.0±0.30
Group II	Cyclophos-phamide	20	7.2±0.36*	6.1±0.34	5.8±0.29
Group III	Ethanolic extract	100	7.5±0.40*	6.2±0.38***	5.6±0.35
Group IV	Ethanolic extract	200	7.4±0.38*	6.1±0.33*	5.5±0.28
Group V	Ethanolic extract	400	7.3±0.36*	6.0±0.32**	5.8±0.30

All values are expressed as mean ± SEM evaluated by ANOVA followed by Dunnet's t test

Figure No:10 Effect Of *Ipomoea Batatus* Stem Extract On RBC Count



7.8 Effect Of *Ipomoea Batatus* Stem Extract On WBC

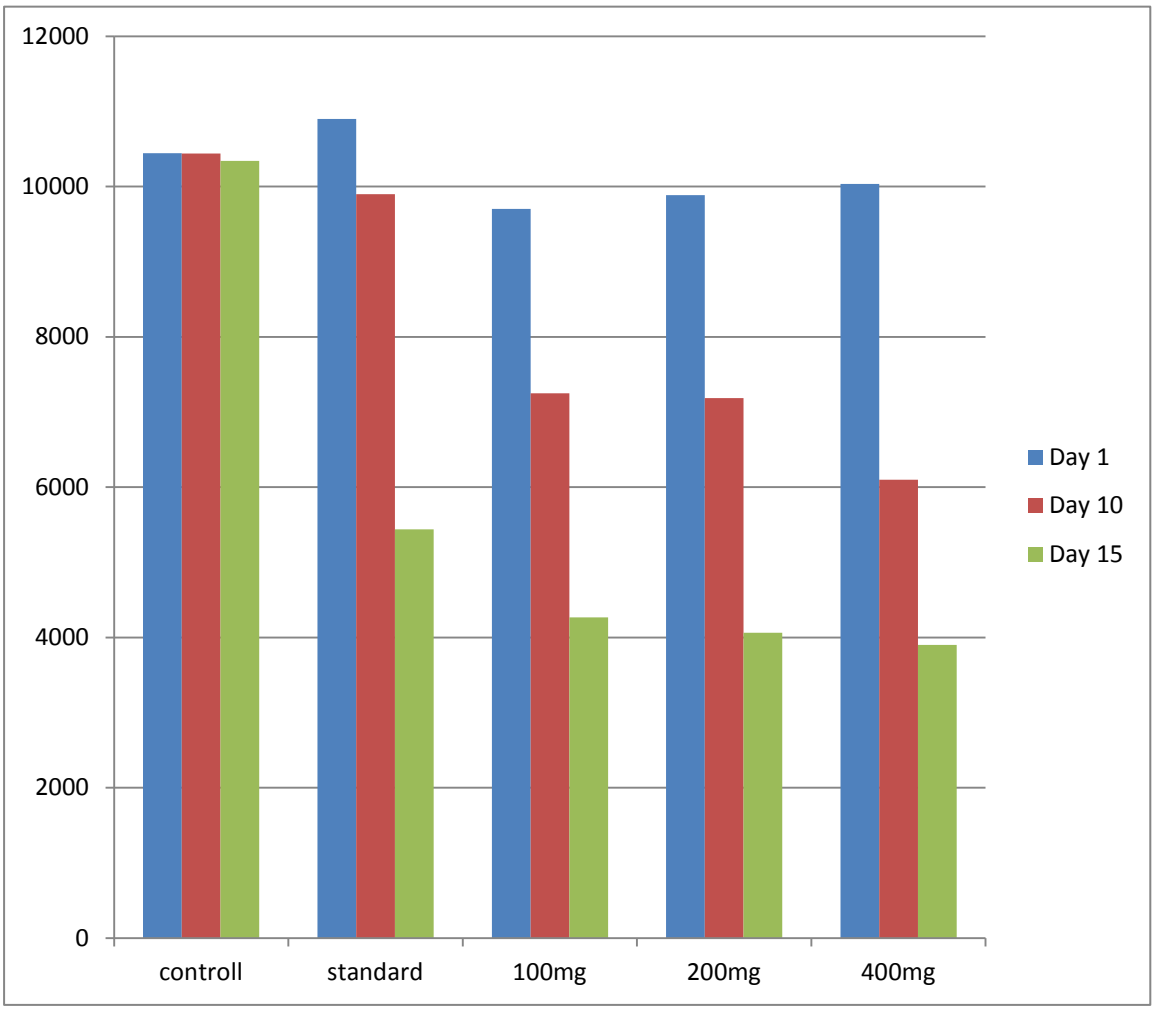
The invivo anticancer activity results have been expressed that the ethanolic extract of stem of ipomoea batatus shown anticancer activity, when compared to control and positive control which used dose levels at 100,200 and 400mg/kg have dose dependent anticancer activity due to constantly decreased WBC cells

Table No:11 Effect Of *Ipomoea Batatus* Stem Extract On WBC

Group N=6	**Treatment**	**Dose (mg/kg body weight)**	**Total WBC count (cells/mm^3)**		
			Day 1	**Day 10**	**Day 15**
Group I	Control	0.6%	10442±8.33	10400±0.004	10343±6.6
Group II	Cyclophosphamide	30	10900±0.001*	9900±0.006*	5440±9.6*
Group III	Ethanolic extract	100	9700±0.007*	7250±0.003*	4265±1.00*
Group IV	Ethanolic extract	200	9883±16.67*	7183±0.65*	4060±1.00*
Group V	Ethanolic extract	400	10033±21.08*	6100±0.004*	3900±0.009*

All values are expressed as mean ± SEM evaluated by ANOVA followed by Dunnet's t test

Figure No: 11 Effect Of Ipomoea Batatus Stem Extract On WBC



Drug concentration vs WBC count

CHAPTER VIII

8.SUMMARY AND CONCLUSION

The preliminary phytochemical investigation revealed the compounds present in the stem of *Ipomoea batatus*. *Invitro* cancer studies were performed using MTT assay with cell lines and found that the material possess significant activity. The LD 50 values were also determined by doing the assay in various concentrations.

Herb based drug industry is growing day by day. The destruction of natural habitats over exploitation and unsustainable harvest has lead to a severe scarcity of raw material. These problems have adversely affected the quantity of herbal drugs.

The current work was specific in the field of oncology and revealed the fact that the medicinal plants are capable of treating cancer

The current work revealed that the stem shows significant anti-cancer activity. So the production of a herbal formulation for the disease will be a boon for the entire pharma industry. The identification of chemical compounds present in the stem of *Ipomoea batatus* which are responsible for the specific activity is remaining. In future work I would like to separate the main constituents that present in the plant, responsible for the action and structure elucidation of those constituents. The present study is an attempt to develop novel plant based anti cancer drugs.

CHAPTER IX

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